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Cotransfection of 293Cre cells with pBHG10lox and a "Lox" shuttle plasmid for generation of Ad expression vectors

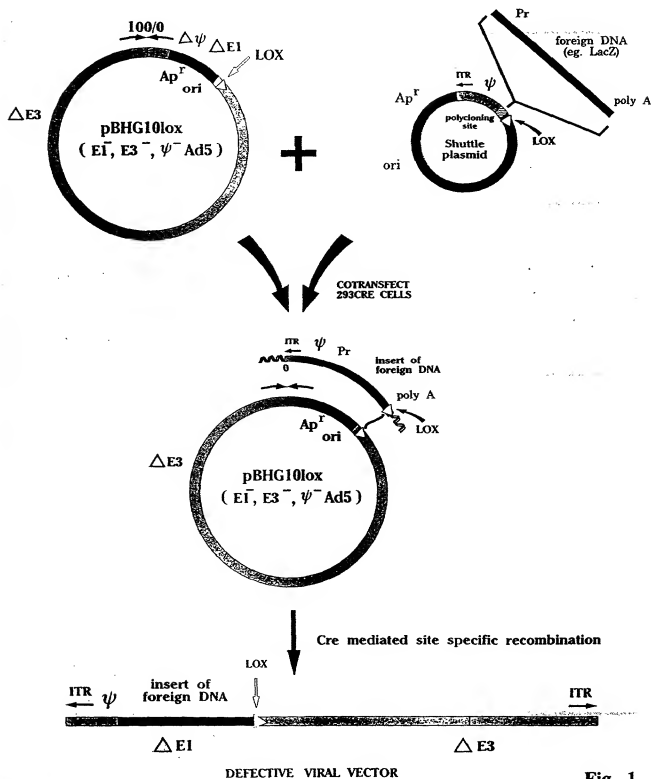


Fig. 1

Cotransfection of 293Cre cells with pBHG10lox and a "lox" shuttle plasmid for generation of Ad expression vectors

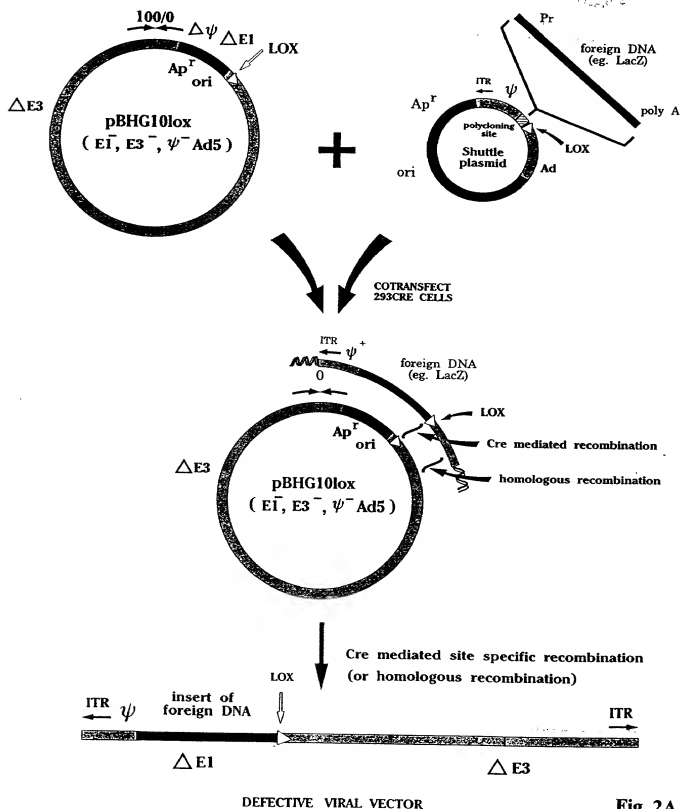


Fig. 2A

CONSTRUCTION OF VARIOUS SHUTTLE PLASMIDS

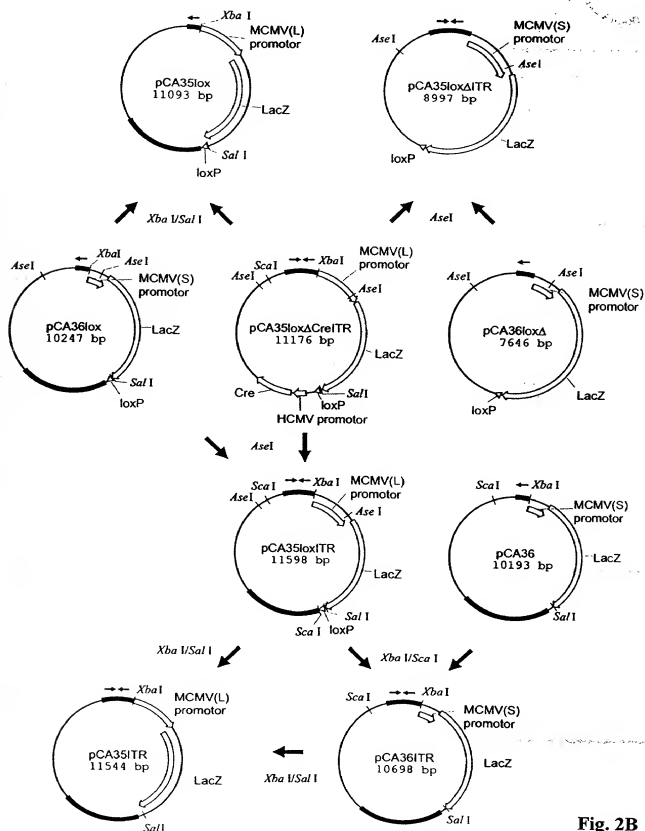


Fig. 2B

OLIGONUCLEOTIDES USED IN CLONING

AB3233/3234 : loxP linker

SEQ. ID. NO. : 1

loxP site

*Bam*H I/*Bgl* II
overhang

5' GATCCAATAACTTCGTATAGCATACATTATACGAAGTTAAAGTACTGAATTCG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATTCATGACTTAAGCCTAG 5'

*Bam*H I/*Bgl* II
overhang

SEQ. ID. NO. : 2

AB14626/14627 : Multiple Cloning Site

SEQ. ID. NO. : 3

Sma I *Bgl* II *Hind* III *Sac* I

Sal I overhang

5' AATTCCTCCCGGGAGATCTAAGCTTGAGCTCG 3'
3' GGGGCCCTCTAGATTGAACTCGAGCAGCT 5'

*Eco*R I overhang

SEQ. ID. NO. : 4

AB6920/6921 : loxP linker

SEQ. ID. NO. : 5

Cla I

Xba I overhang

5' CTAGCAATAACTTCGTATAGCATACATTATACGAAGTTATATCGATG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATAGCTACGATC 5'

Xba I overhang

SEQ. ID. NO. : 6

AB14680/14681 : loxP linker

SEQ. ID. NO. : 7

Cla I

Bsp I overhang

5' TGACAATAACTTCGTATAGCATACATTATACGAAGTTATATCGATG 3'
3' GTTATTGAAGCATATCGTATGTAATATGCTTCAATATAGCTACACT 5'

Bsp I overhang

SEQ. ID. NO. : 8

CONSTRUCTION OF A CIRCULAR GENOMIC PLASMID FOR Ad VECTOR RESCUE USING THE Cre/ loxP SYSTEM

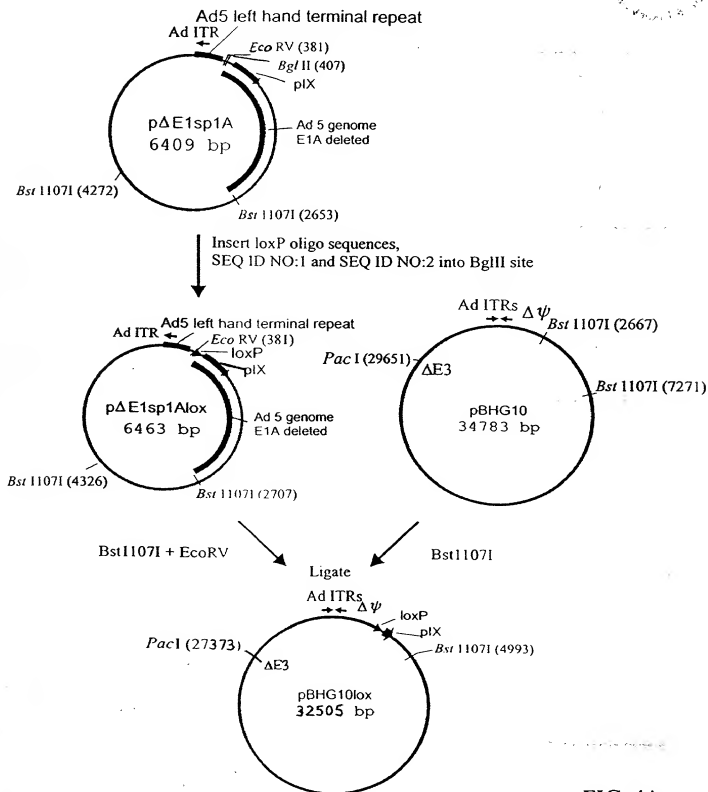


FIG. 4A

CONSTRUCTION OF pBHGdX1Plox

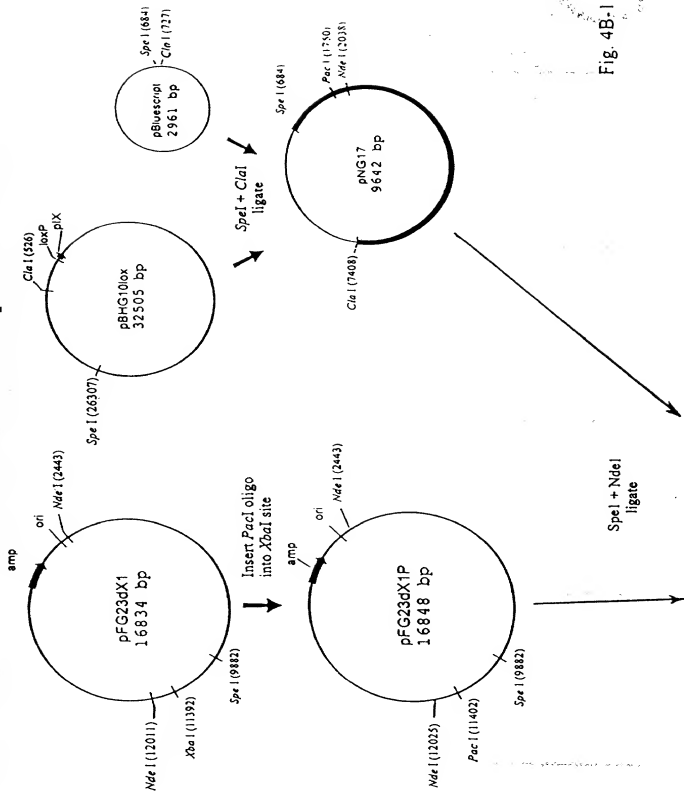


Fig. 4B-1

CONSTRUCTION OF pBHGX1Plox

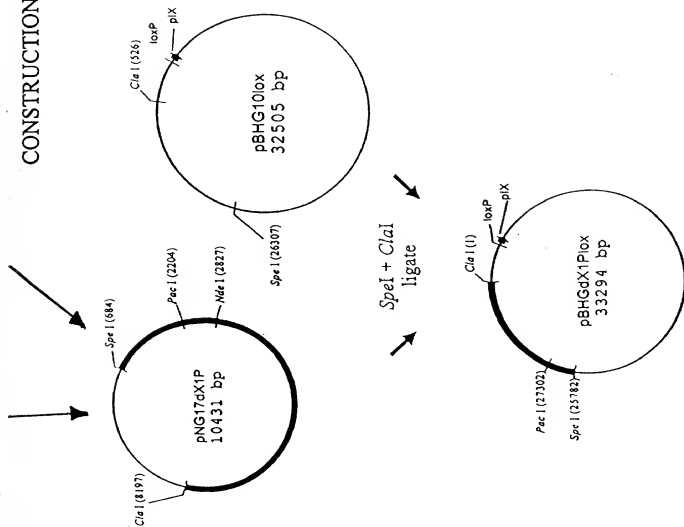


Fig. 4B-2

CONSTRUCTION OF pBHGE3lox

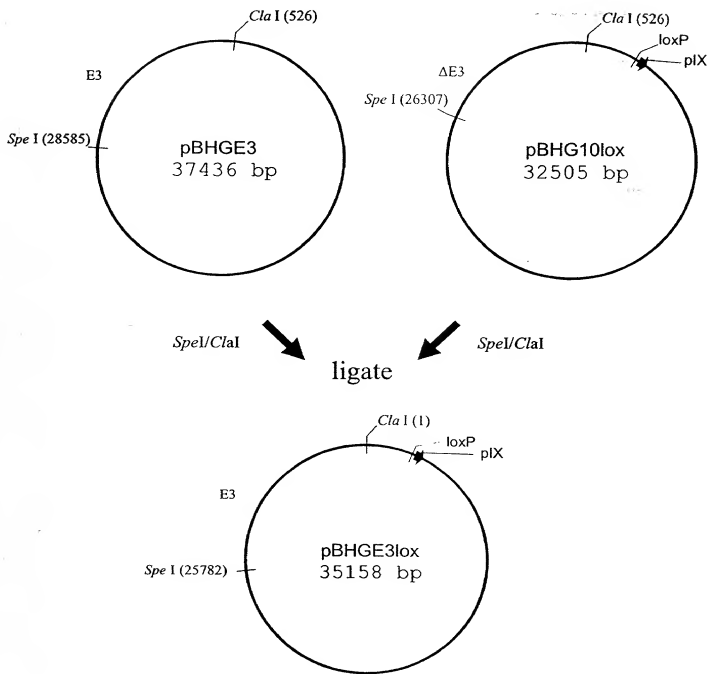


Fig. 4C

CONSTRUCTION OF Ad GENOMIC PLASMIDS ENCODING CRE

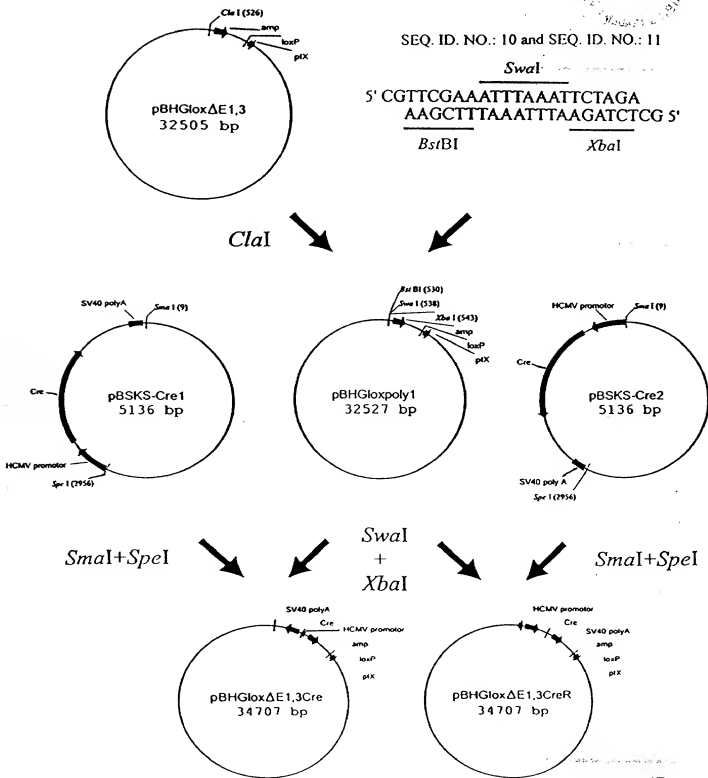


Figure 4D

CONSTRUCTION OF pΔE1SP1A & pΔE1SP1B loxP PLASMIDS FOR RESCUE OF FOREIGN DNA

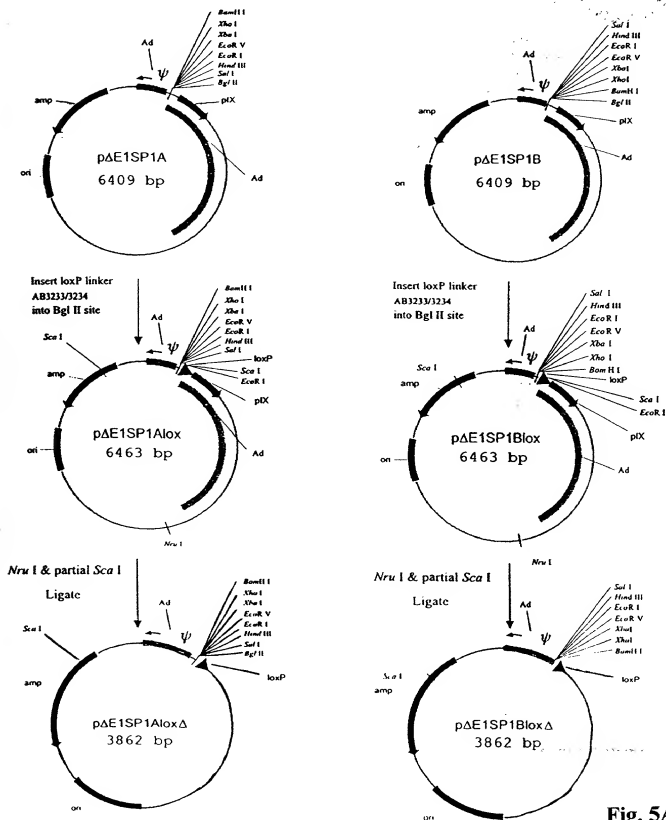


Fig. 5A

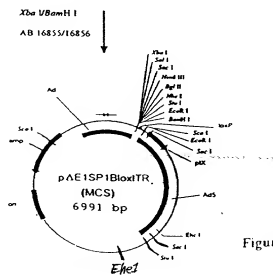
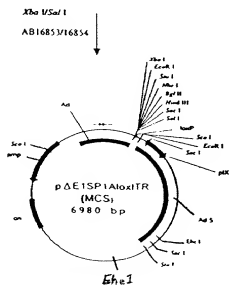
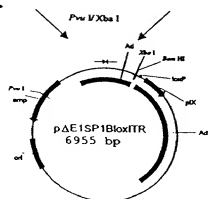
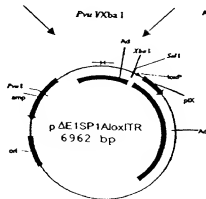
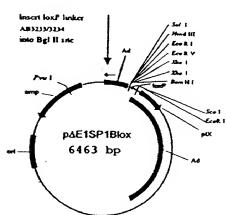
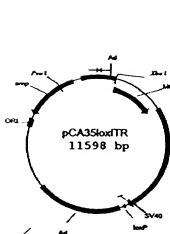
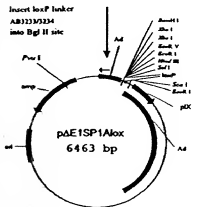
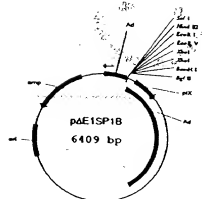
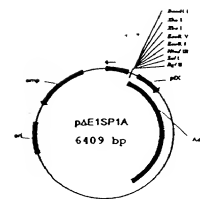


Figure 5B

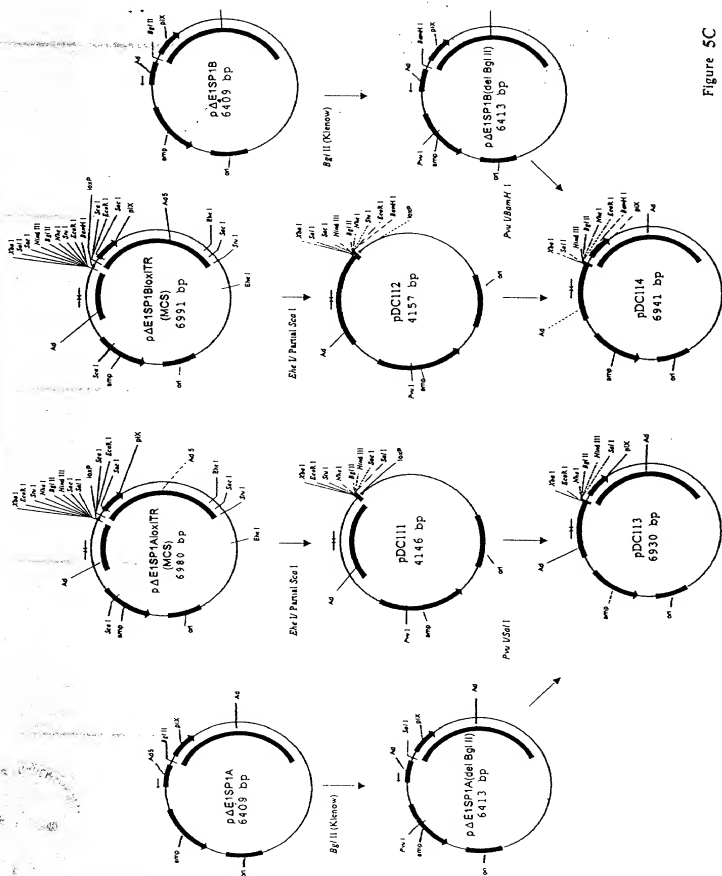


Figure 5C

SEQ. ID. NO.: 12 (AB16853) and SEQ. ID. NO.: 13 (AB16854)



SEQ. ID. NO.: 14 (AB16855) and SEQ. ID. NO.: 15 (AB16856)

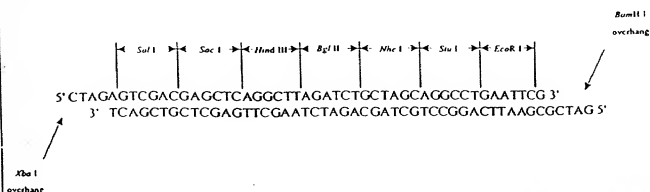
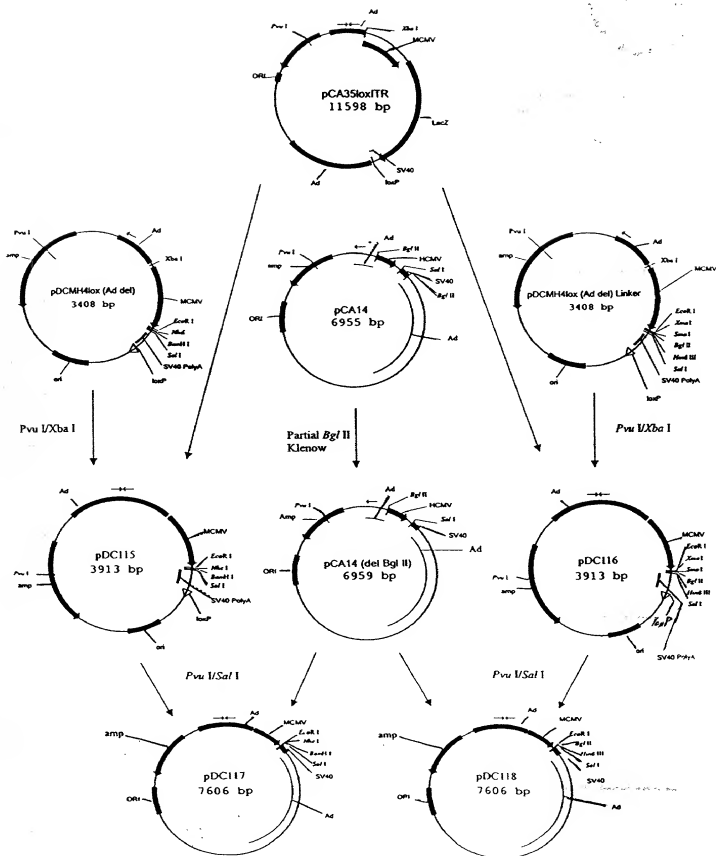


Figure 5D



CONSTRUCTION OF pMH4LOX, pMH4LOX Δ and pMH4LOX Δ LINK SHUTTLE PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

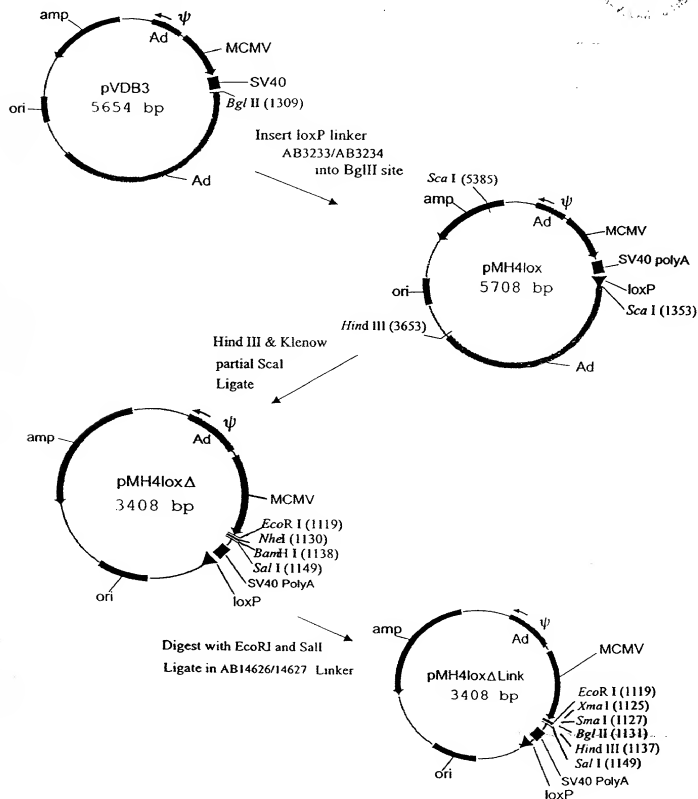


Fig. 6A

CONSTRUCTION OF A SHUTTLE PLASMID CONTAINING A pUC DERIVED ORIGIN

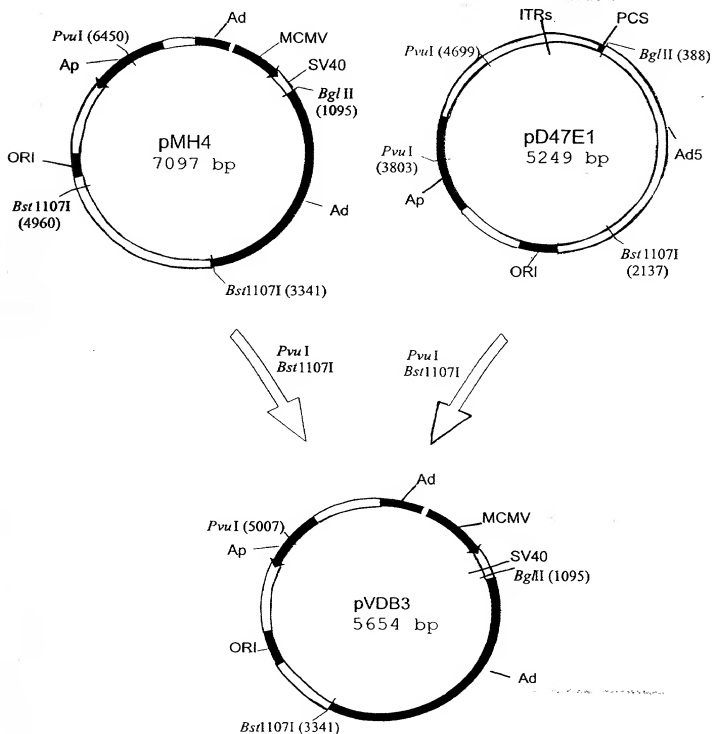


Fig. 6B

CONSTRUCTION OF HCMV loxP PLASMIDS FOR RESCUE OF EXPRESSION CASSETTES

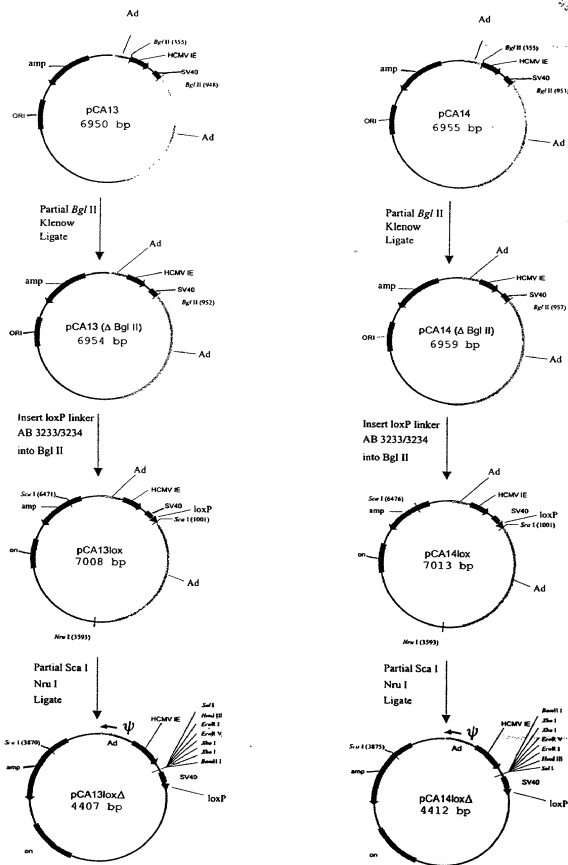


Fig. 7

CONSTRUCTION OF pCA36LOX and pCA36LOX Δ SHUTTLE PLASMIDS FOR RESCUE OF LACZ

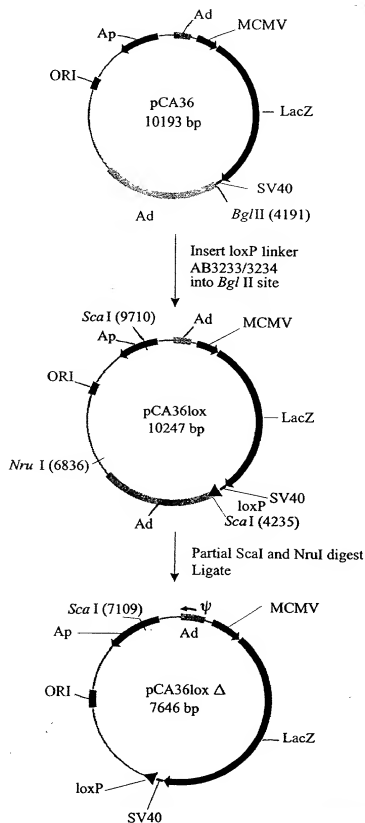


Fig. 8A

Cotransfection of 293Cre cells with AdLC8c DNA-TP and a shuttle plasmid containing a loxP site for generation of Ad expression vectors

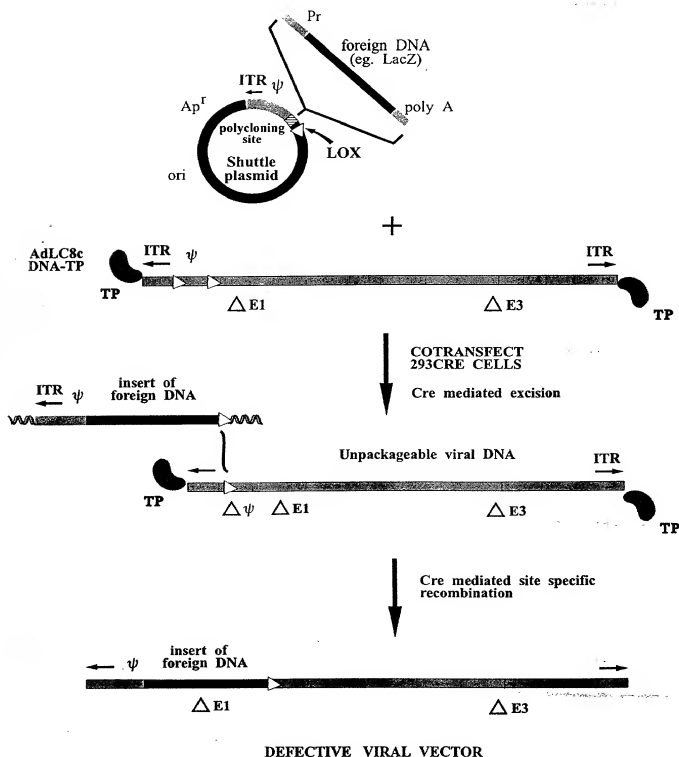
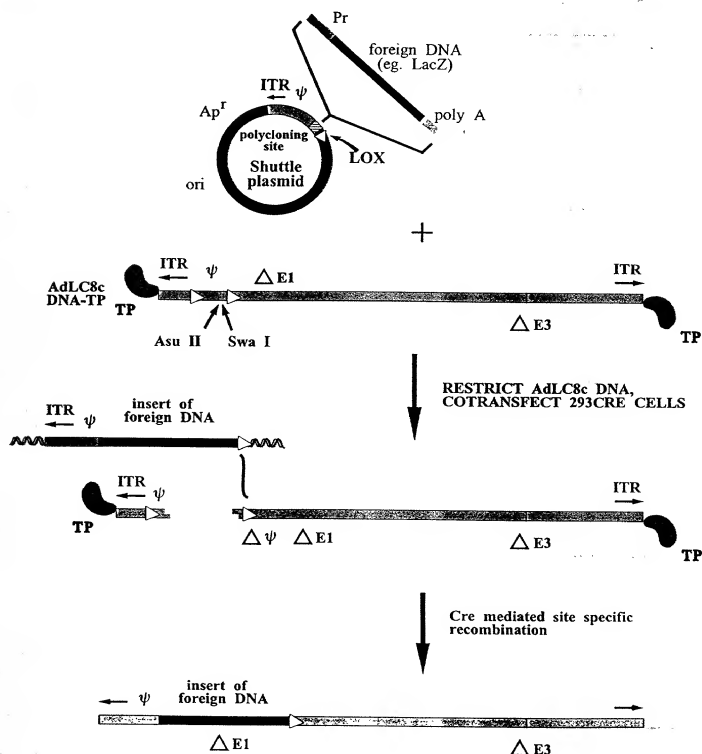


Fig. 8B

Cotransfection of 293Cre cells with restricted AdLC8c DNA-TP and loxP shuttle plasmid for generation of Ad expression vectors



DEFECTIVE VIRAL VECTOR

Fig. 8C

CONSTRUCTION OF SHUTTLE PLASMIDS EXPRESSING Cre

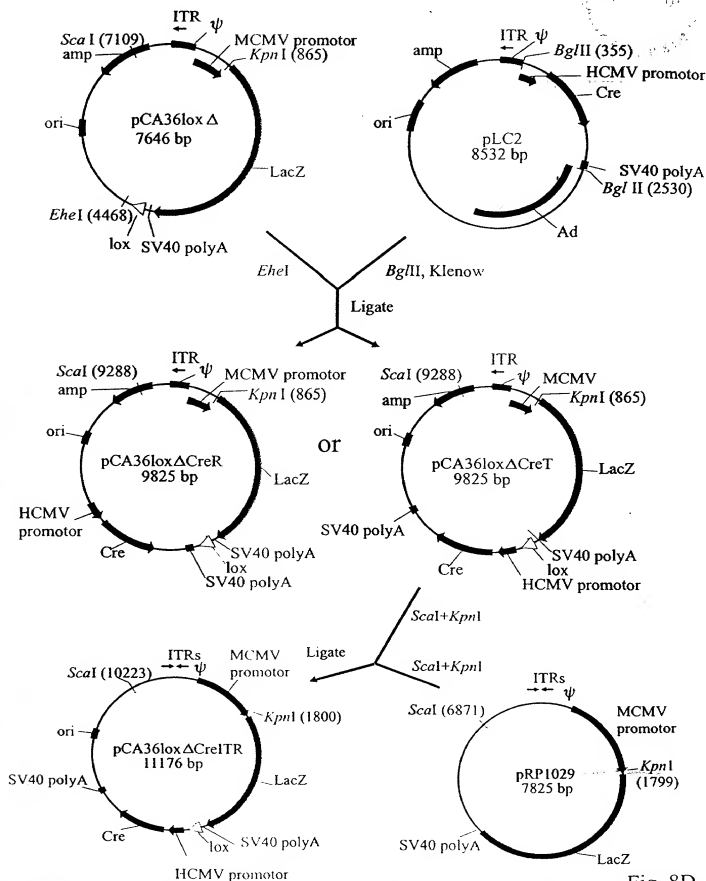


Fig. 8D

CONSTRUCTION OF Ad GENOMIC PLASMID ENCODING CRE

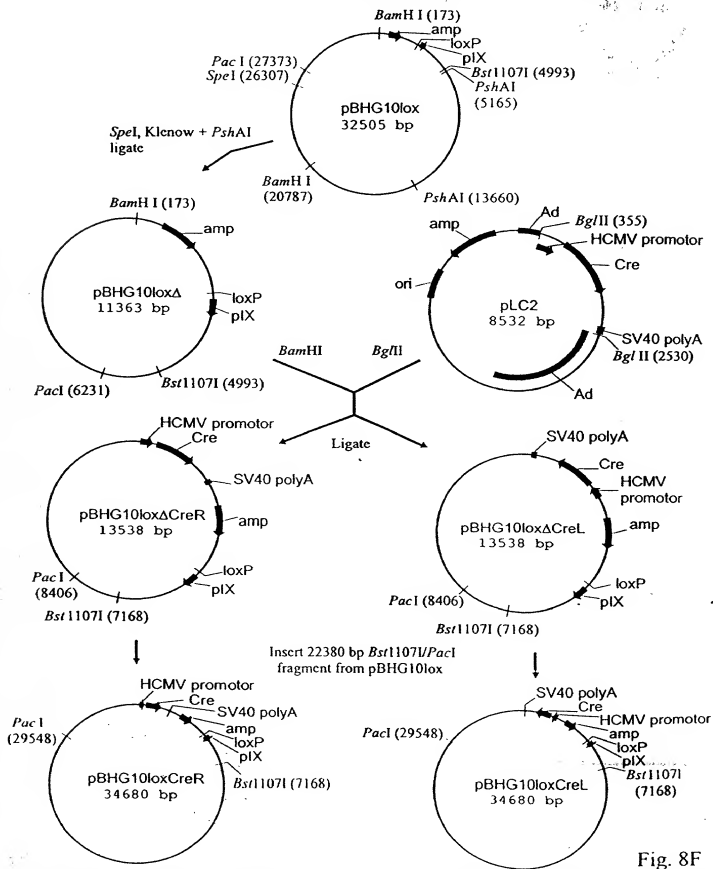


Fig. 8F

CONSTRUCTION OF pAB14lox Δ

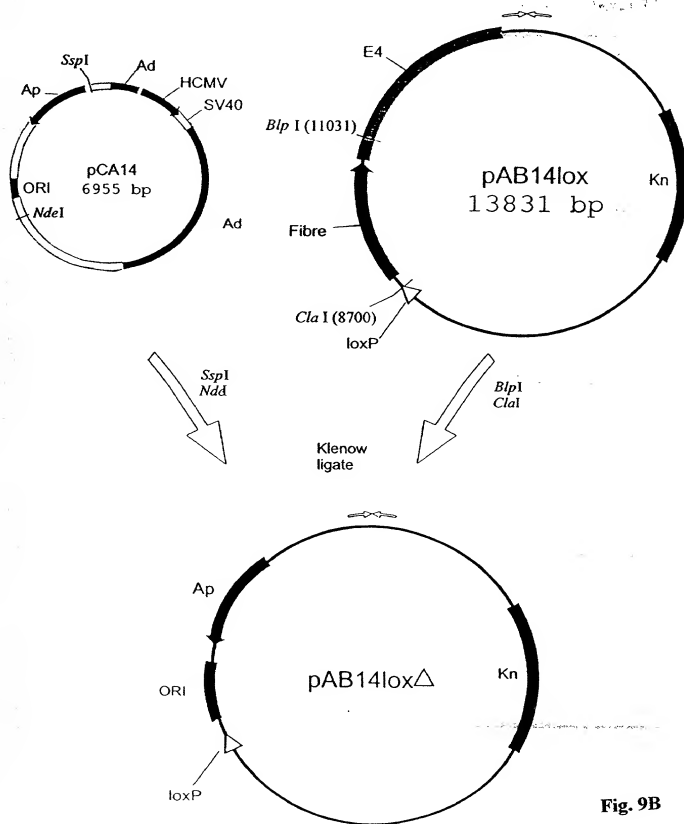
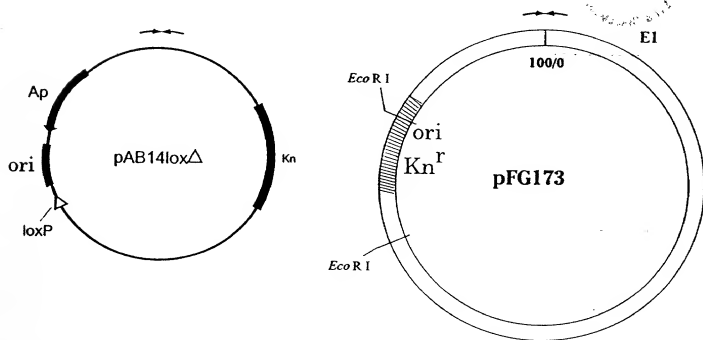


Fig. 9B

CONSTRUCTION OF pFG173lox



Restriction, transformation of *E. coli*,
homologous recombination

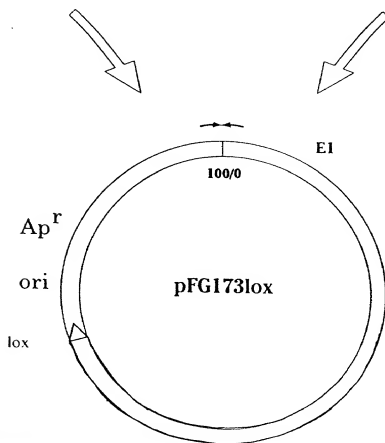


Fig. 9C

CONSTRUCTION OF pFG23dX1lox AND pFG23dX1lox_c FOR RESCUE OF MUTANT FIBRE INTO AD VIRUS

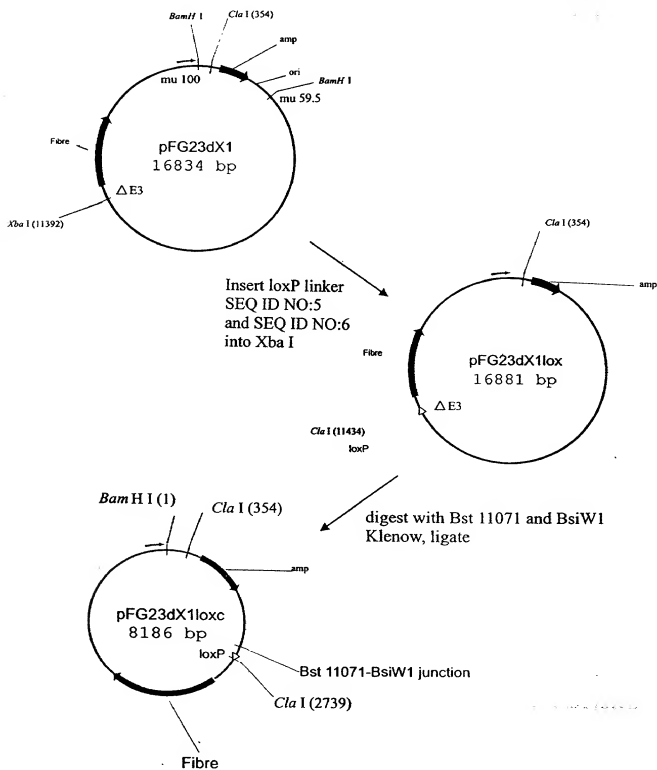


Fig. 10

A PLASMID FOR RESCUE OF A FOREIGN DNA INTO AD VIRUS

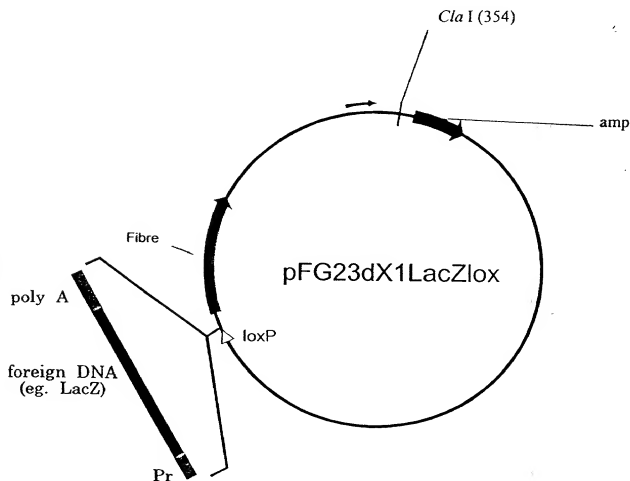
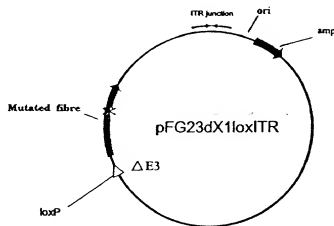
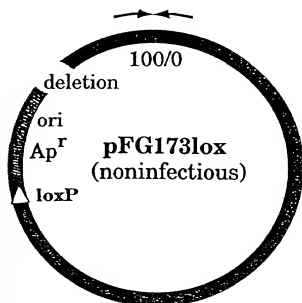


Fig. 11A

RESCUE OF FIBRE MUTATIONS USING CRE/LOX RECOMBINATION



COTRANSFECTION OF 293CRE CELLS
SITE-SPECIFIC RECOMBINATION



NONDEFECTIVE ($E1^+$) VIRUS WITH MUTATED FIBRE GENE

Fig. 11B

Isolation of a virus containing a mutant fibre gene by Cre-lox recombination using DNA-TP and cotransfection

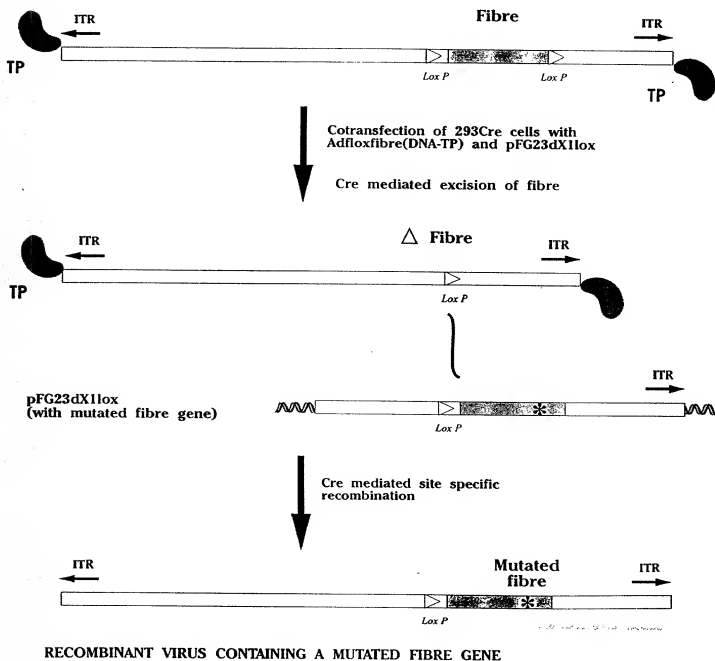
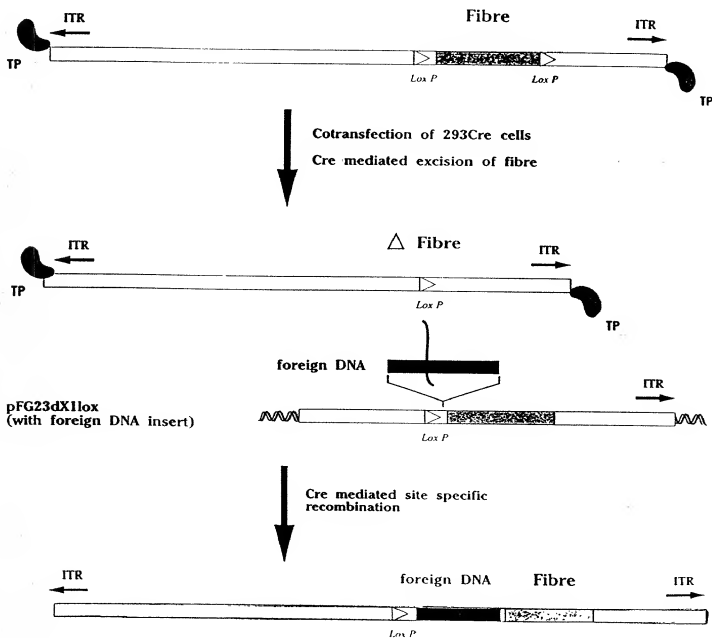


Fig. 12

Isolation of a virus containing a foreign DNA insert upstream of the fibre gene by Cre-lox recombination



RECOMBINANT VIRUS CONTAINING AN INSERT OF FOREIGN DNA
UPSTREAM OF THE FIBRE GENE

Fig. 13

CONSTRUCTION OF pAB14FL0X FOR ISOLATION OF AN AD VIRUS WITH A FLOXED FIBRE GENE

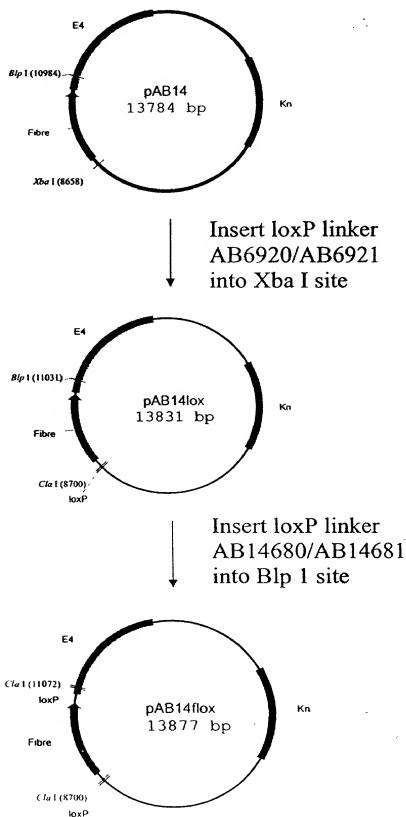


Fig. 14

Cotransfection of 293 cells with pBHGI0lox and a "Lox" shuttle plasmid expressing Cre for generation of Ad expression vectors

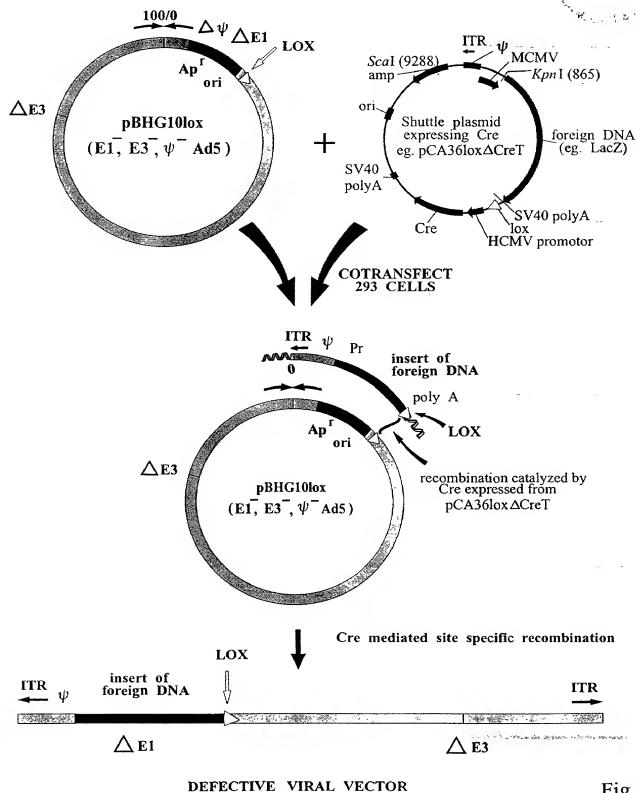


Fig. 8E